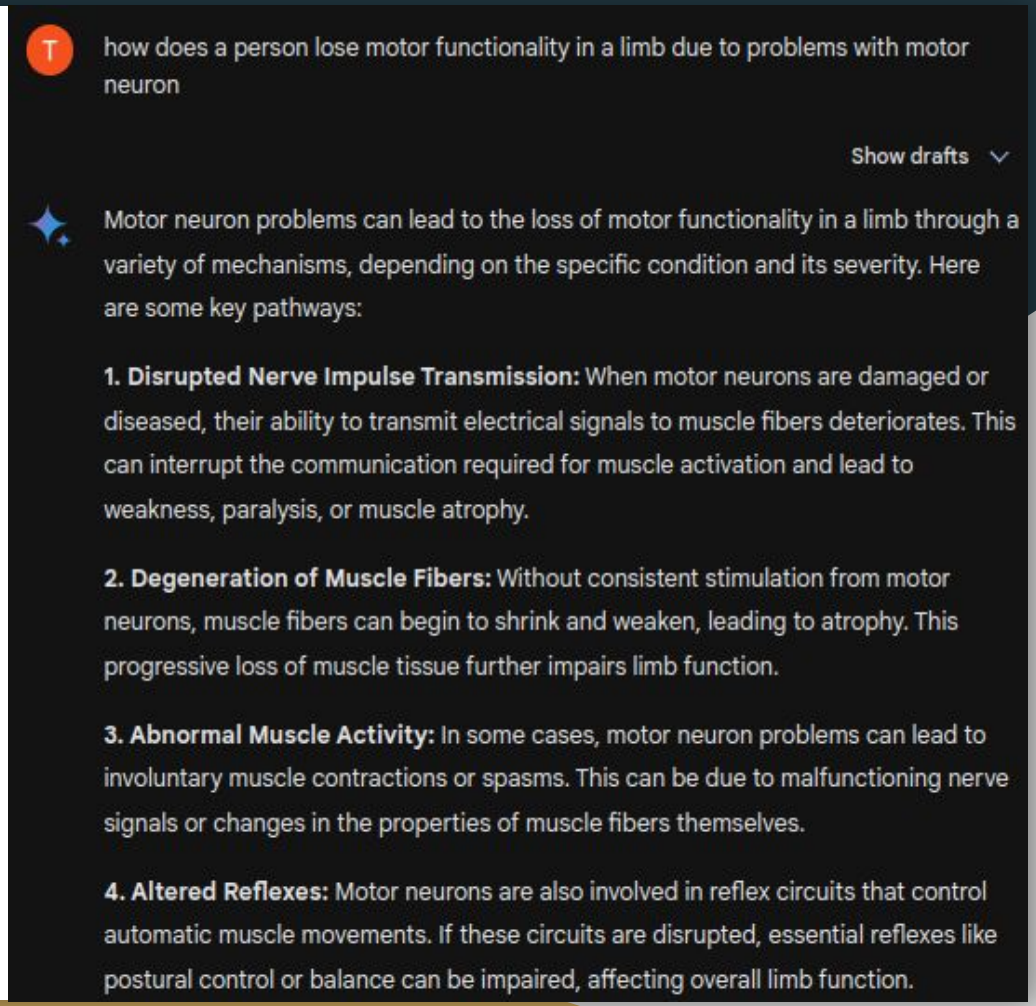
Therapeutic Cure for the regain of Muscular Function using Optogenetically engineered *Bacillus subtilis* producing Acetylcholine

By Tuhin Kumar Raut

Why this project?

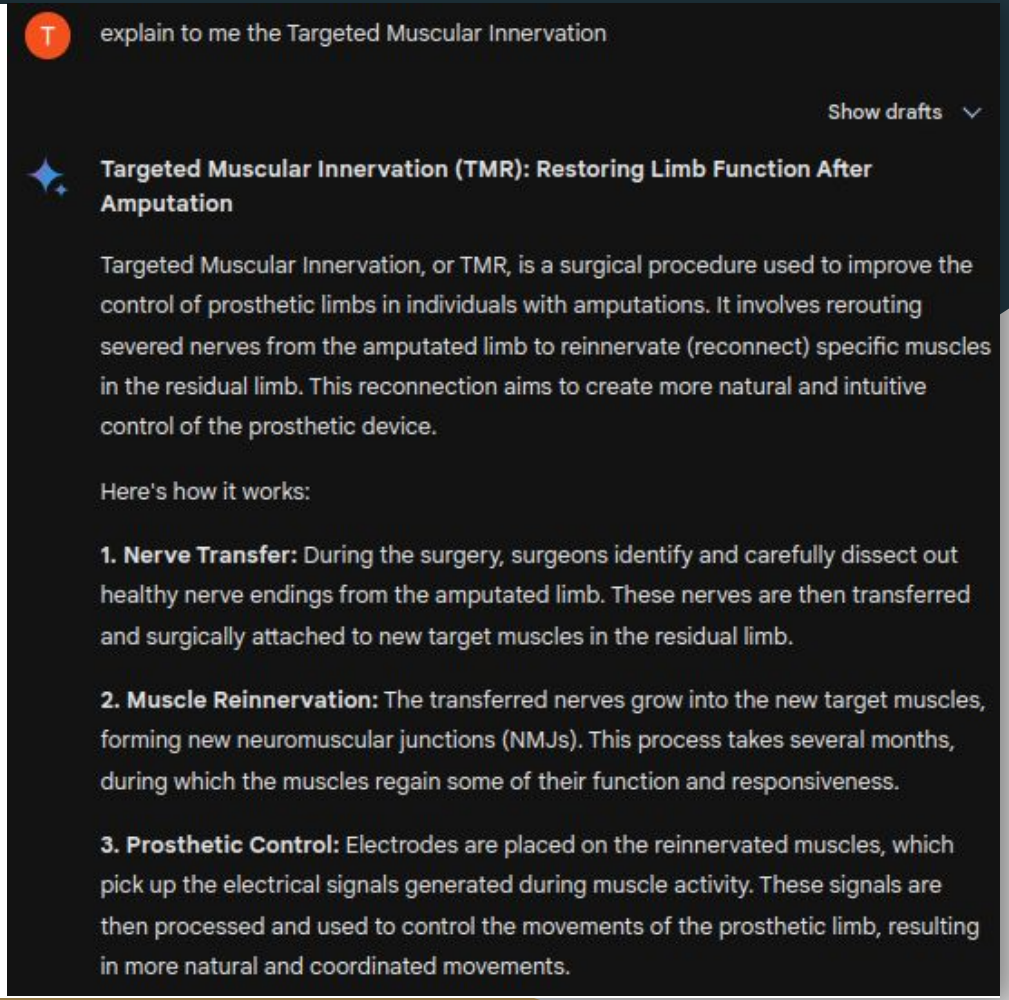
There has been an ever existing problem in Elderly and those undergoing limb re-attachment surgeries that, people lose control over some regions of their muscles.

Of which one of the most prevalent reasons is the disrupted Nerve Impulse Transmission due to death or severing of a nerve cell or a collection of cells.



What are the existing solutions?

TMI or Targetted Muscular Innervation is one of the most successful and prevalent solutions to bring back the functionality of the muscles.



Why not just use electrodes to stimulate the muscles?

Technique known as Neuromuscular Electrical Stimulation exists, which is capable of triggering contraction of muscles through electrical impulses, but still it doesn’t restore natural functionality of muscle contraction and NMES lacks specificity, which prevents its use for fine movements, which TMI and our proposed technique are capable of producing.

So, why does TMI exist despite NMES?

- **Precision and Control:** TMI offers superior precision and control, allowing for more natural movement patterns and potentially restoring complex functions like grasping or fine motor control, which are difficult to achieve with NMES.
- **Long-Term Benefits:** While NMES might be helpful for temporary muscle strengthening or pain management, TMI has the potential for more permanent functional improvements and sustained muscle growth.
- **Addressing Nerve Damage:** For cases where nerve damage prevents natural muscle activation, TMI offers a unique solution by rerouting healthy nerves, while NMES cannot bypass damaged pathways.

Problems with TMI

It is very very costly. (~$32-36k)

The level of function regained through the procedure is very less

Failed Targeted Muscle Reinnervation: Findings at Revision Surgery and Concepts for Success

John M. Felder, MD,[✉1] Stahs Pripotnev, MD,[1] Ivica Ducic, MD, PhD,[1] Rachel Skladman, MD,[1] Austin Y. Ha, MD,[1] and Mitchell A. Pet, MD[1]

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Background: Go to: ▸

Although it was initially described for improved myoelectric control, targeted muscle reinnervation (TMR) has quickly gained popularity as a technique for neuroma control. With this rapid increase in utilization has come broadening indications and variability in the described technique. As a result, it becomes difficult to interpret published outcomes. Furthermore, there is no literature discussing the management of failed cases which are undoubtedly occurring.

Methods:

This is a retrospective case series of two patients who underwent revision surgery for failed TMR. The authors also review the current literature on TMR and outline technical and conceptual pitfalls and pearls based on our local experience.

Results:

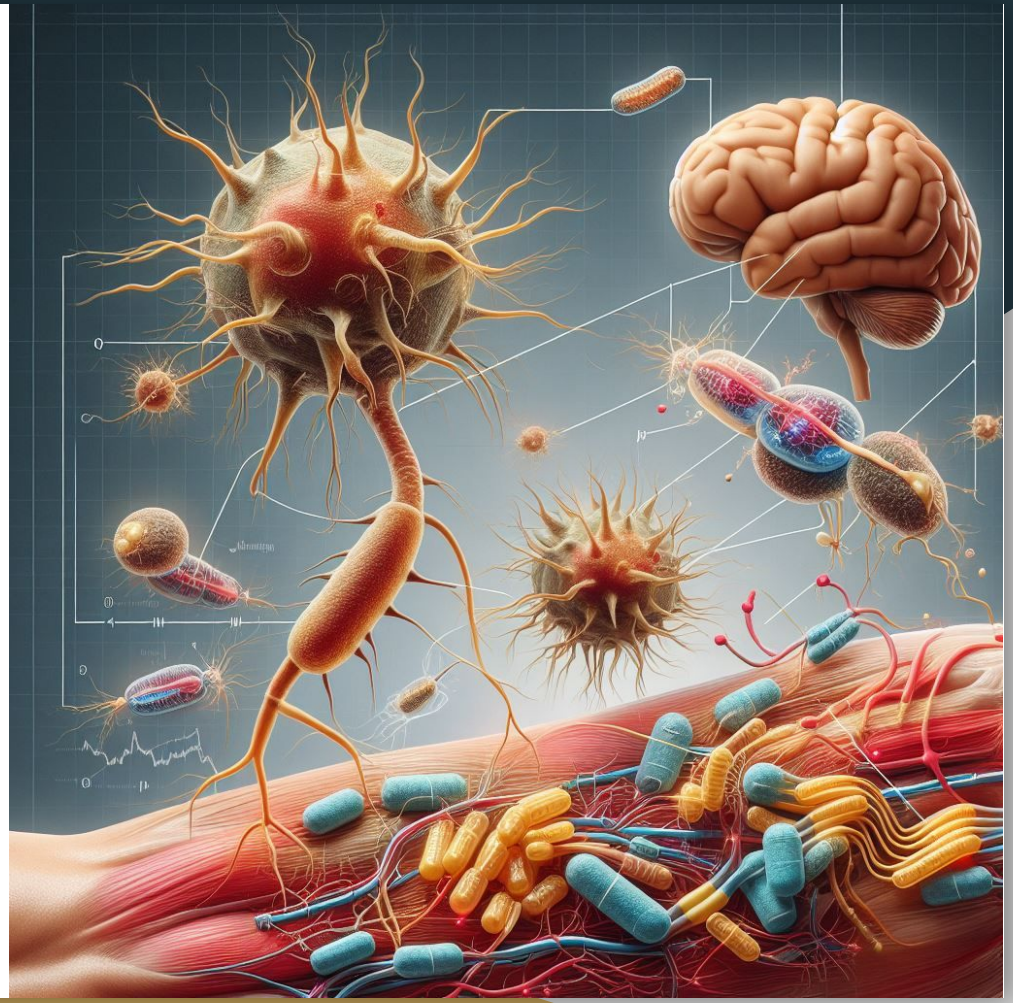
Excessive donor nerve redundancy, kinking, donor–recipient nerve size mismatch, superficial placement of the nerve coaptation, inappropriate target selection, and incomplete target muscle denervation were identified as technical pitfalls of TMR surgery. Techniques to avoid these pitfalls were described.

Conclusions:

Although TMR has been a major development in amputee care for both pain management and improved myoelectric control, it is important to acknowledge that it is not a foolproof surgery and does not provide a guaranteed result. Failed cases of TMR represent opportunities to learn about factors contributing to unfavorable outcomes and refine our techniques empirically.

Proposed Solution

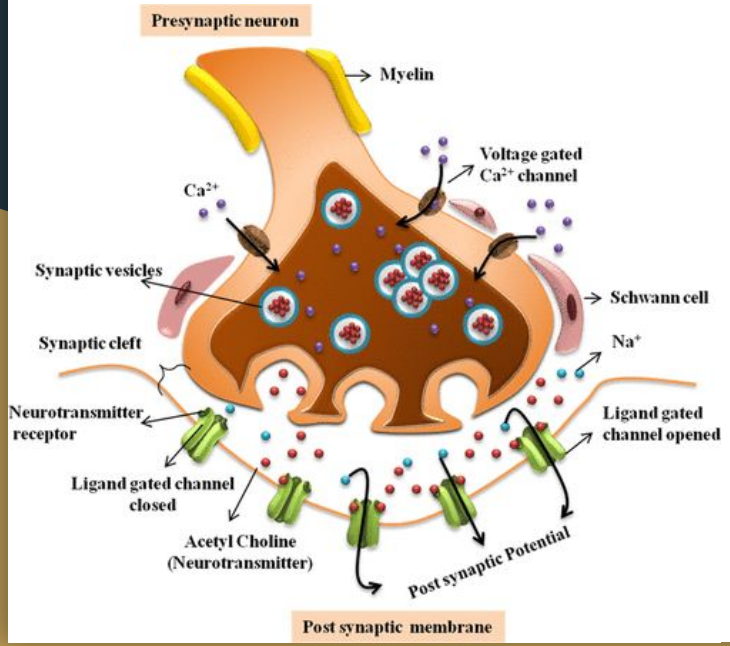
Replacing the need for neurons, for motor function, with an encapsulated collection of Optogenetically modified *Bacillus subtilis* producing Acetylcholine.



Important components in the Project

1. Microcapsule creation and Biocompatible microLEDs embedded in the capsule
2. Overexpression of Acetylcholine expressing genes induced by light
3. HTI for controlling the population inside capsule
4. Converting EEG signals to Light signals by a central hub.

How does the Neuromuscular Junction function



Here's a shorter explanation of muscle contraction at the NMJ:

Action in the brain sends a signal to a motor neuron.

Neuron fires, releasing acetylcholine (ACh) at the NMJ.

ACh binds to muscle receptors, opening channels and letting sodium in.

Sodium rush depolarizes the muscle, triggering calcium release.

Calcium binds to proteins, allowing myosin heads to grip actin filaments.

Myosin heads "row" on actin, shortening the muscle and causing contraction.

Acetylcholinesterase breaks down ACh, calcium gets pumped away, and the muscle relaxes.

Details on the individual components of the project

1. **Capsule Formation**

Hydrogel based porous capsule embedded with microLEDs should do the trick. (but not sure)

Can think of easier alternatives after consulting Prof. Prashant Mishra

Dry lab team would be generating a 3-D model first for the exact construction of the capsule.

MicroLED neural probe for effective *in vivo* optogenetic stimulation

Hiroki Yasunaga, Hibiki Takeuchi, Koyo Mizuguchi, Atsushi Nishikawa, Alexander Loesing, Mikiko Ishikawa, Chikako Kamiyoshihara, Susumu Setogawa, Noriaki Ohkawa, and Hiroto Sekiguchi

Details on the individual components of the project

2. Overexpression of Acetylcholine Genes

Bacillus subtilis has been shown to produce acetylcholine naturally, so we need to make optogenic modifications to the TF of the genes to overexpress that protein formation for that amount of time.

3.1.3. Acetylcholine

Acetylcholine is a common cholinergic neurotransmitter in vertebrates and insects that functions as a local mediator in the central and peripheral nervous systems by transducing excitatory signals between neurons [54]. Its dysregulation is closely associated with neurodegenerative diseases such as AD [55,56]. Acetylcholine was first discovered in a study of ergot on wheat rye in the early 1900s, although it was only later noticed that *Bacillus acetylcholini* rather than ergot actually produced this neurochemical [40]. Since then, acetylcholine has been found to be produced by multiple bacteria including *Lactobacillus plantarum*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* [39,41]. Notably, *B. subtilis* contains larger quantities of acetylcholine than *E. coli* or *S. aureus* [41]. As acetylcholine cannot cross the blood–brain barrier, neurons in the central nervous system synthesize acetylcholine from choline and acetyl coenzyme A catalyzed by choline acetyltransferase [57]. Peripherally derived choline can be transported to the brain via the carriers located on capillary endothelial cells [58].

Ubiquitous expression of acetylcholine and its biological functions in life forms without nervous systems

Koichiro Kawashima [a], Hidemi Misawa [a], Yasuhiro Moriwaki [a], Yoshihito X. Fujii [a], Takeshi Fujii [a], Yoko Horiuchi [a], Tomoya Yamada [a], Tadayuki Imanaka [b], Masahiro Kamekura [c]

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Abstract

Using a radioimmunoassay (RIA) with high specificity and sensitivity (1 pg/tube) for acetylcholine (ACh), we have been able to measure the ACh content in samples from the bacteria, archaea and eucarya domains of the universal phylogenetic tree. We found detectable levels of ACh to be ubiquitous in bacteria (e.g., *Bacillus subtilis*), archaea (e.g., *Thermococcus kodakaraensis* KOD1), fungi (e.g., shiitake mushroom and yeast), plants (e.g., bamboo shoot and fern) and animals (e.g., bloodworm and lugworm). The levels

The work of the dry lab team would be to simulate the kinetics of the reaction and provide specific duration and wavelength of light to the cells, to fine tune the amount to be produced.

Details on the individual components of the project

3. HTI based kill switch for Controlling Bacterial Population

HTIs have shown to be effective in killing Gram Positive bacteria and bacteria aren't able to evolve to generate resistance towards this particular mechanism of Antimicrobial effect.

The dry lab team would do a similar job as to what described in the prev page for this as well.

Hemithioindigo-Based Visible Light-Activated Molecular Machines Kill Bacteria by Oxidative Damage

Ana L. Santos, Alexis van Venrooy, Anna K. Reed, Aaron M. Wyderka, Víctor García-López, Lawrence B. Alemany, Antonio Oliver, George P. Tegos, James M. Tour

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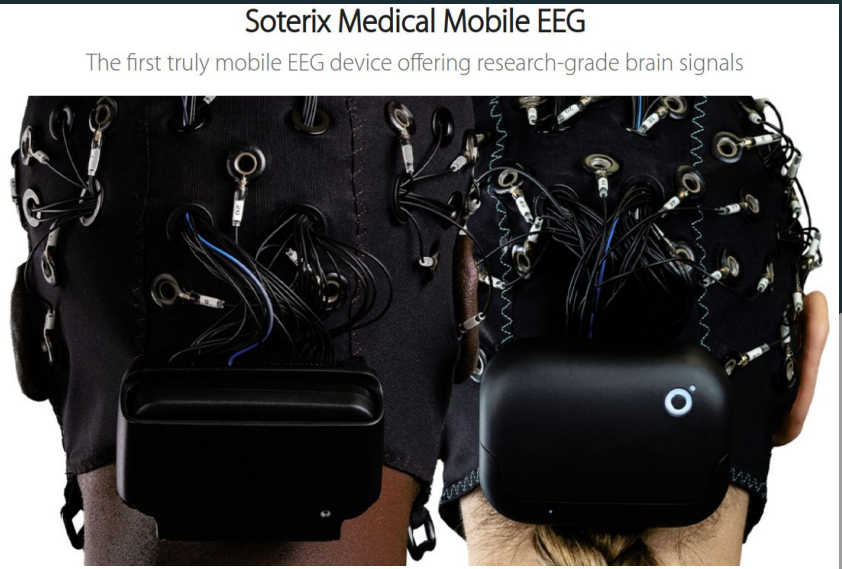
Abstract

Antibiotic resistance is a growing health threat. There is an urgent and critical need to develop new antimicrobial modalities and therapies. Here, a set of hemithioindigo (HTI)-based molecular machines capable of specifically killing Gram-positive bacteria within minutes of activation with visible light (455 nm at 65 mW cm^{-2}) that are safe for mammalian cells is described. Importantly, repeated exposure of bacteria to HTI does not result in detectable development of resistance. Visible light-activated HTI kill both exponentially growing bacterial cells and antibiotic-tolerant persister cells of various Gram-positive strains, including methicillin-resistant *S. aureus* (MRSA). Visible light-activated HTI also eliminate biofilms of *S. aureus* and *B. subtilis* in as little as 1 h after light activation. Quantification of reactive oxygen species (ROS) formation and protein carbonyls, as well as assays with various ROS scavengers, identifies oxidative damage as the underlying mechanism for the antibacterial activity of HTI. In addition to their direct antibacterial properties, HTI synergize with conventional antibiotics in vitro and in vivo, reducing the bacterial load and mortality associated with MRSA infection in an invertebrate burn wound model. To the best of the authors' knowledge, this is the first report on the antimicrobial activity of HTI-based molecular machines.

Details on the individual components of the project



4. EEG signal to Light signal

Portable EEG devices have been developed

Wireless transfer the EEG interpreted data to the central hub

Central hub to transfer the data using wires or wirelessly to the microcapsules

Can take help from Prof. Sandeep Kumar, from Bharti School, for the study of EEG as he has done extensive work on this topic.

EEG-Datasets

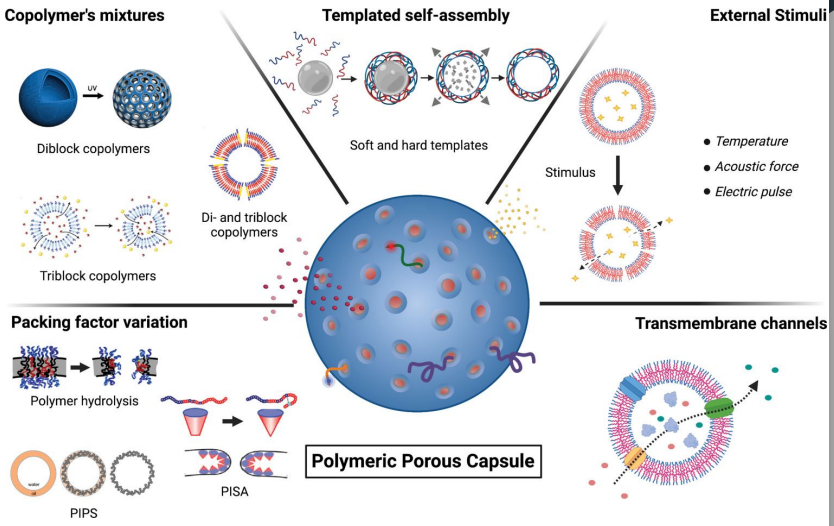
Left/Right **Hand** 1D/2D **movements**: 19-electrode data of one subject with various combinations of 1D and 2D **hand movements** (actual execution). Imagination of Right ...

What we should be doing for iGEM in the time frame

For iGEM we mostly need to provide a proof of concept, mainly in Therapeutics Village.

I know we won't be able to prove the efficacy in real muscle tissue, but using it to demonstrate the contraction in a collection of cells would be sufficient as a proof of concept, in my opinion.

And if we are not able to encapsulate the bacteria into the nanostructure due to technical difficulties, we could just show the light stimulated bacteria producing acetylcholine are able to make muscle cells contract. And make a 3D model of how the capsule would look like and function, which is quite common in most of the projects, even winners.



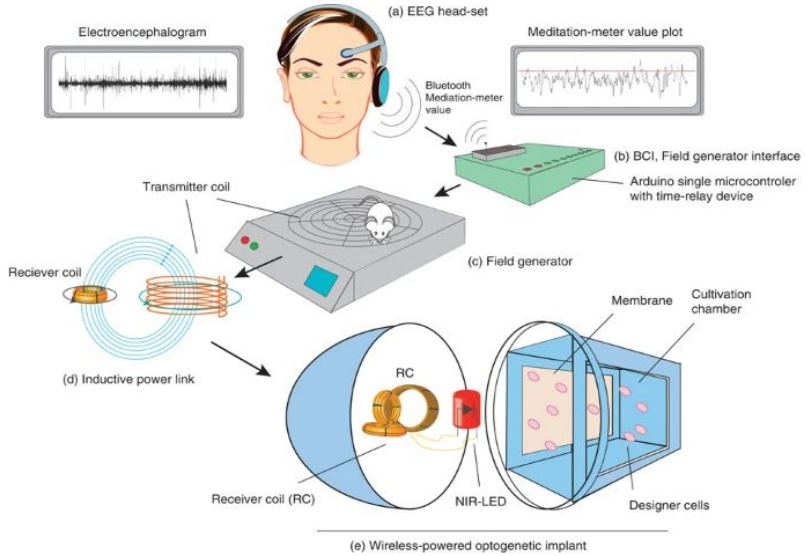
A similar work

Martin Fussenegger et. al. have performed an experiment on optogenetic control of a gene expression using EEG-BCI methodology. But they have genetically modified mammalian cells and also haven't targeted Neuron-Muscle interaction.

But we can use the similar proof of concept experiment they did to test their hypothesis for our project as well.

This also gives some hope of future implementation of our project.

Figure 5: Schematic representation of mind-controlled transgene expression.



The mind-controlled transgene expression device consisted of (**a**) an EEG headset that captured brain-wave activities (the encephalogram), identified mental state-specific electrical patterns (biofeedback, concentration, meditation) and processed discrete meditation-meter values (0–100; meditation-meter value plot), which were transmitted via Bluetooth to (**b**) the Arduino single-board microcontroller with a time-relay device and switching the (**c**) field generator ON and OFF. This BCI (**a**–**c**) controlled (**d**) the TC (**c**,**d**) of the field generator, which inductively coupled with the (**d**,**e**) receiver coil (RC) of the (**e**) wireless-powered optogenetic implant. (**e**) The NIR light LED illuminated the culture chamber of the wireless-powered optogenetic implant and programmed the designer cells to produce SEAP, which diffused through the semi-permeable membrane. The blood SEAP levels of mice with subcutaneous wireless-powered optogenetic implants containing designer cells that were freely moving on the field generator could be modulated by the human subject's mindset in a wireless, remote-controlled manner. (See Supplementary Fig. 5 for a schematic of the electronic components).

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